

University of Groningen

## Fecal microbiota composition and frailty

van Tongeren, SP; Slaets, JPJ; Harmsen, HJM; Welling, GW

*Published in:*  
Applied and environmental microbiology

*DOI:*  
[10.1128/AEM.71.10.6438-6442.2005](https://doi.org/10.1128/AEM.71.10.6438-6442.2005)

**IMPORTANT NOTE:** You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

*Document Version*  
Publisher's PDF, also known as Version of record

*Publication date:*  
2005

[Link to publication in University of Groningen/UMCG research database](#)

*Citation for published version (APA):*

van Tongeren, SP., Slaets, JPJ., Harmsen, HJM., & Welling, GW. (2005). Fecal microbiota composition and frailty. *Applied and environmental microbiology*, 71(10), 6438-6442.  
<https://doi.org/10.1128/AEM.71.10.6438-6442.2005>

### Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

### Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

*Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.*

## Fecal Microbiota Composition and Frailty

Sandra P. van Tongeren,<sup>1</sup> Joris P. J. Slaets,<sup>2</sup> H. J. M. Harmsen,<sup>1</sup>  
and Gjalb W. Welling<sup>1\*</sup>

Department of Medical Microbiology<sup>1</sup> and Department of Geriatrics,<sup>2</sup> University Medical  
Center Groningen, Hanzeplein 1, 9713 GZ Groningen, The Netherlands

Received 17 February 2005/Accepted 2 June 2005

**The relationship between fecal microbiota composition and frailty in the elderly was studied. Fecal samples from volunteers with high frailty scores showed a significant reduction in the number of lactobacilli (26-fold). At much higher population levels, both the *Bacteroides/Prevotella* (threefold) and the *Faecalibacterium prausnitzii* (fourfold) groups showed a significant reduction in percentage of total number of hybridizable bacteria in the elderly with high frailty scores. In contrast to this, the number of *Enterobacteriaceae* was significantly higher (sevenfold) in samples from very frail volunteers.**

The gut microbiota forms an essential part of a complex ecosystem that plays an important role in human health and nutrition (5, 8, 34). Bacterial colonization of the gastrointestinal (GI) tract is affected by various factors such as host, microbiological, physiological, dietary, and environmental factors. Studies of babies (10) and adults (6, 12, 37) have shown that after birth the gut microbiota composition keeps evolving, even in old age (23).

Knowledge about elderly gut microbiota composition is far from complete (3, 13, 16, 17, 27). Changes in gut microbiota composition related to ageing may have implications for the health of elderly persons and may be caused by factors which include psychosocial stress, mobility, and nutrition. Psychosocial stress factors may contribute to the development of anorexia or changes in the immune system, which may in turn affect the gut microbiota composition (15, 19). Small bowel motor pattern changes have been reported with ageing, and in addition, the degree of mobility of the elderly person may influence bowel motility. Reduced bowel movement has a negative effect on digestion but also causes constipation and may therefore be associated with changes in the gut microbiota.

Frailty comprises several of the above-mentioned factors (28, 30). Frailty can generally be defined as a state of decreasing reserves with respect to those functions and resources that are essential for a person to maintain an acceptable level of functioning. The Groningen Frailty Indicator (GFI) (28, 30) is a short 15-item questionnaire aiming to identify patients that have diminished reserves in one or more of the core domains of functioning. These domains are: mobility, physical fitness, comorbidity, weight loss, vision, hearing, cognition, and psychosocial resources. Of all these domains it has empirically been shown that these are associated with a variety of adverse outcomes. The current view on the clinical measurement of frailty encompasses the whole person's functioning and physiology, emphasizing the interaction of physical and psychosocial systems (25). The GFI builds on the assumption that strong

interaction effects often exist between different weaknesses of older patients, reinforcing each other into a downward spiral of further decline.

Because it is often difficult to separate different problems in causal order in elderly patients with multiple problems, it is deemed possible to identify the core domains of functioning and summate the affected domains because each of them contributes to the latent variable frailty. Psychometric analyses of the GFI in different development studies have shown that it is a one-dimensional (with principal component analysis) and internally consistent scale (KR-20 = 0.71) (28). With respect to the validity and predictive value of the GFI in hospital patients and in general practitioners' patients, it has been shown that the GFI is able to predict adverse outcome in different groups of patients (cancer patients, emergency room admissions, osteoporotic fractures, surgery). All studies showed that the GFI was a better predictor than calendar age.

In this paper, we describe the fecal microbiota composition in relation to frailty. Advances in the field of molecular phylogeny have made it possible to study bacterial populations by a culture-independent approach (1, 6, 9, 11, 18, 31, 35, 36). The predominant microbiota composition of fecal samples of volunteers was determined with fluorescent in situ hybridization (12), and the level of frailty was determined by the GFI (28).

Fresh fecal samples from 23 elderly volunteers (aged 70 to 100 years, with a median of 86 years) were collected. All volunteers were living in the Heymans Elderly Center, Groningen, The Netherlands. The study was compliant with the Medical Research Involving Human Subjects Act and informed consent was obtained. Samples came from elderly of two different levels of care in this center, varying from relatively little to complete nursing care. All volunteers were supplied with the same food menu. At least 4 weeks prior to collection of the samples, the volunteers did not receive antibiotics. Samples were stored at 4°C immediately after collection and processed within 24 h.

The total amount of cells and the total number of bacteria and specific bacterial groups of each fecal sample were enumerated as described previously (12), using the set of probes listed in Table 1. The level of frailty of the volunteers was determined using the GFI questionnaire (28, 30). One point

\* Corresponding author. Mailing address: Department of Medical Microbiology, University Medical Center Groningen, Hanzeplein 1, 9713 GZ Groningen, The Netherlands. Phone: 31 50 3633513. Fax: 31 503633528. E-mail: g.w.welling@med.umcg.nl.

TABLE 1. Fecal microbiota composition of elderly volunteers with low and high frailty scores as determined by DAPI staining (total cells) and hybridization with bacterial probes

| Bacterial group or parameter                      | Stain or probe          | Reference      | Low-frailty group ( <i>n</i> = 13)          |                          |                     |        | High-frailty group ( <i>n</i> = 10)         |                          |                     |        |
|---|-------------------------|----------------|---|--------------------------|---------------------|--------|---|--------------------------|---------------------|--------|
|   |                         |                | Median no. of cells/g of feces <sup>a</sup> |                          | Median % microbiota |        | Median no. of cells/g of feces <sup>a</sup> |                          | Median % microbiota |        |
|   |                         |                | Dry wt                                      | Wet wt                   | DAPI                | Eub338 | Dry wt                                      | Wet wt                   | DAPI                | Eub338 |
| Total cells                                       | DAPI                    |                |   |                          |                     |        |   |                          |                     |        |
| Total bacteria                                    | Eub338                  | 2              | 2.0 × 10 <sup>11</sup>                      | 5.3 × 10 <sup>10</sup>   | 100                 | 100    | 2.0 × 10 <sup>11</sup>                      | 6.6 × 10 <sup>10</sup>   | 100                 | 100    |
| <i>Bacteroides/Prevotella</i> group               | Bac303                  | 22             | 1.3 × 10 <sup>11</sup>                      | 3.5 × 10 <sup>10</sup>   | 53.9                | 24.2*  | 1.1 × 10 <sup>11</sup>                      | 3.4 × 10 <sup>10</sup>   | 54.2                | 9.4*   |
| <i>E. rectale/C. coccoides</i> group              | Erec482                 | 6              | 2.7 × 10 <sup>10</sup>                      | 7.7 × 10 <sup>9</sup>    | 11.0                | 19.7   | 8.7 × 10 <sup>9</sup>                       | 2.9 × 10 <sup>9</sup>    | 4.5                 | 13.2   |
| <i>Ruminococcus</i> group                         | Rfla729/Rbro730         | 12             | 1.6 × 10 <sup>10</sup>                      | 5.1 × 10 <sup>9</sup>    | 10.6                | 15.2   | 1.3 × 10 <sup>10</sup>                      | 4.1 × 10 <sup>9</sup>    | 6.7                 | 23.8   |
| <i>Atopobium</i> group                            | Ato291                  | 11             | 1.9 × 10 <sup>10</sup>                      | 4.5 × 10 <sup>9</sup>    | 6.3                 | 3.4    | 2.9 × 10 <sup>10</sup>                      | 7.9 × 10 <sup>9</sup>    | 12.7                | 10.6   |
| <i>Faecalibacterium prausnitzii</i> group         | Fpra645                 | 32             | 5.3 × 10 <sup>9</sup>                       | 1.3 × 10 <sup>9</sup>    | 2.1                 | 3.1*   | 9.0 × 10 <sup>9</sup>                       | 2.4 × 10 <sup>9</sup>    | 4.3                 | 0.7*   |
| <i>Eubacterium cylindroides</i> group             | Ecy387                  | 12             | 2.2 × 10 <sup>9</sup>                       | 6.1 × 10 <sup>8</sup>    | 1.2                 | 1.4    | 4.6 × 10 <sup>8</sup>                       | 1.6 × 10 <sup>8</sup>    | 0.3                 | 0.9    |
| <i>Bifidobacterium</i>                            | Bifi64                  | 21             | 1.5 × 10 <sup>9</sup>                       | 4.9 × 10 <sup>8</sup>    | 0.9                 | 1.3    | 1.6 × 10 <sup>9</sup>                       | 4.2 × 10 <sup>8</sup>    | 0.8                 | 0.5    |
| <i>Streptococcus</i> group                        | Strc493                 | 6              | 2.0 × 10 <sup>9</sup>                       | 4.6 × 10 <sup>8</sup>    | 0.9                 | 1.1    | 4.6 × 10 <sup>8</sup>                       | 1.4 × 10 <sup>8</sup>    | 0.2                 | 0.8    |
| <i>Lactobacillus/Enterococcus</i> group           | Lab158                  | 9              | 1.1 × 10 <sup>9</sup>                       | 3.8 × 10 <sup>8</sup>    | 0.7                 | 0.3**  | 8.7 × 10 <sup>8</sup>                       | 2.8 × 10 <sup>8</sup>    | 0.4                 | 0.04** |
| <i>Clostridium</i> group                          | Chs150/Clit135          | 6              | 3.1 × 10 <sup>8</sup> *                     | 9.8 × 10 <sup>7</sup> ** | 0.1*                | 0.2    | 2.5 × 10 <sup>7</sup> *                     | 7.1 × 10 <sup>6</sup> ** | 0.03*               | 0.02   |
| <i>Enterobacteriaceae</i>                         | Ecol1531 <sup>23s</sup> | 24             | 2.4 × 10 <sup>8</sup>                       | 5.4 × 10 <sup>7</sup>    | 0.1                 | 0.01   | 1.8 × 10 <sup>7</sup>                       | 5.8 × 10 <sup>6</sup>    | 0.01                | 0.6    |
| <i>Veillonella</i>                                | Vei1223                 | 12             | 6.6 × 10 <sup>7</sup> *                     | 2.1 × 10 <sup>7</sup> *  | 0.05*               | 0.1    | 4.7 × 10 <sup>8</sup> *                     | 1.4 × 10 <sup>8</sup> *  | 0.3*                | 0.01   |
| <i>Phascolarctobacterium</i> group                | Phasco741               | 12             | 9.2 × 10 <sup>6</sup>                       | 2.7 × 10 <sup>6</sup>    | 0.005               | 0.001  | 1.6 × 10 <sup>7</sup>                       | 4.9 × 10 <sup>6</sup>    | 0.01                | 0.004  |
| Sum of specific probes                            |                         |                | 4.3 × 10 <sup>5</sup>                       | 1.0 × 10 <sup>5</sup>    | 0.0002              | 0.001  | 4.1 × 10 <sup>6</sup>                       | 1.3 × 10 <sup>6</sup>    | 0.002               | 60.6   |
| <i>Lachnospira</i> group                          | Lach571                 | 12             | 2.0 × 10 <sup>8</sup>                       | 4.5 × 10 <sup>7</sup>    | 34.0                | 0.2    | 1.5 × 10 <sup>7</sup>                       | 4.0 × 10 <sup>6</sup>    | 30.3                | 0.01   |
| <i>Eubacterium hallii</i> group                   | Ehal1469                | 12             | 1.3 × 10 <sup>8</sup>                       | 3.3 × 10 <sup>7</sup>    | 0.1                 | 0.1    | 9.9 × 10 <sup>7</sup>                       | 2.7 × 10 <sup>7</sup>    | 0.1                 | 0.1    |
| <i>Enterococcus faecium/Enterococcus faecalis</i> | Enfm2/Enf3              | — <sup>b</sup> | 3.6 × 10 <sup>5</sup>                       | 1.0 × 10 <sup>5</sup>    | 0.0002              | 0.001  | 1.8 × 10 <sup>6</sup>                       | 5.0 × 10 <sup>5</sup>    | 0.001               | 0.002  |

<sup>a</sup> The data were analyzed assuming that when no bacteria of the target group were detected, the bacterial number was 1.0 × 10<sup>5</sup> per gram, wet weight. \*, statistically significant (*P* ≤ 0.05, two-sided); \*\*, statistically significant (*P* ≤ 0.01, two-sided).

<sup>b</sup> K. Waar, personal communication.

could be scored on each question. Mobility (four questions) and psychosocial factors (five questions) are the major components in the total frailty score. In some cases the questions about mobility and psychosocial factors were answered by the medical staff or the relatives of the elderly. The volunteers were divided into two groups, one ( $n = 13$ ) with a low frailty score (1 to 4) and one ( $n = 10$ ) with a high frailty score (5 or more) (30). Both groups consisted mainly of females, with two males in each group.

The results were statistically analyzed by SPSS for Windows, release 10.0.5, standard version. Analysis with Spearman's rank-order correlation coefficient showed that the age and frailty scores were not significantly related (correlation coefficient = 0.058). To compare the enumeration results of fecal samples of the groups with low and high frailty scores, the Mann-Whitney U test was applied. The enumeration results are listed in Table 1. The total number of cells was the same in both frailty groups, i.e.,  $2.0 \times 10^{11}$  cells/g (dry weight). Of the total cells, the percentage of total hybridizable bacterial cells was approximately 54%. With group-specific probes, 34.0% and 30.3% of the total number of cells and 69.9% and 60.6% of the total number of hybridizable bacteria in the low- and high-frailty volunteer groups could be accounted for, respectively.

The percentages of hybridizable bacteria of the three largest groups of the fecal microbiota of healthy (low frailty scores) elderly, i.e., *Bacteroides/Prevotella*, *Eubacterium rectale/Clostridium coccoides*, and *Ruminococcus* (type species *R. flavefaciens*) were 24.2, 19.7, and 15.2%, respectively. The same groups in healthy adults aged 20 to 55 yrs were rather similar, 27.7, 22.7, and 10.3%, respectively (12). Larger differences were observed when these three percentages were compared to those of the fecal microbiota of elderly with high frailty scores, 9.4, 13.2, and 23.8%, respectively.

More differences in fecal microbiota composition can be observed between elderly persons with low and high frailty scores. The most predominant were the *Bacteroides/Prevotella*, *E. rectale/C. coccoides*, *Ruminococcus*, *Atopobium*, and *F. prausnitzii* bacterial groups. Of the total number of cells, the percentages of three of these groups were decreased in the high-frailty volunteers, i.e., *Bacteroides/Prevotella* (from 11.0 to 4.5%), *E. rectale/C. coccoides* (from 10.6 to 6.7%), and *F. prausnitzii* (from 1.2 to 0.3%). In contrast to this, the percentages of the *Ruminococcus* (from 6.3 to 12.7%) and *Atopobium* (from 2.1 to 4.3%) groups were increased in the high-frailty volunteers.

Four groups of bacteria were significantly different between fecal samples from the two volunteer groups, i.e., those that hybridized with the Lab158, Ecoli1531 (*Enterobacteriaceae*), Bac303 (*Bacteroides/Prevotella*), and Fprau645 (*F. prausnitzii*) probes. *Lactobacillus* numbers were obtained by subtracting those obtained with the Enfm2/Enf13 probes from those determined with the Lab158 probe. The number of lactobacilli was  $3.0 \times 10^8$  cells/g (dry weight) in low-frailty volunteers and  $1.1 \times 10^7$  cells/g (dry weight) in high-frailty volunteers ( $P = 0.005$ ). Of the total number of cells the percentage of lactobacilli was 0.1% in low-frailty volunteers and 0.01% in high-frailty volunteers ( $P = 0.005$ ). Additionally, of the total number of hybridizable bacteria the percentage of lactobacilli was 0.3% in low-frailty volunteers and 0.02% in high-frailty volunteers ( $P =$

0.004). As analyzed by Spearman's rho, the number of lactobacilli showed a significant negative correlation with the GFI (correlation coefficient =  $-0.694$ ,  $P < 0.0001$ ).

At much higher population levels, both the *Bacteroides/Prevotella* and *F. prausnitzii* group showed a significant reduction in percentage of the total number of hybridizable bacteria for the high-frailty volunteers. The *F. prausnitzii* group showed a significant reduction in percentage from 3.1% for the low-frailty volunteers to 0.7% for the high-frailty volunteers ( $P = 0.03$ ). Analysis with Spearman's rho showed a strong correlation between the *F. prausnitzii* group and lactobacilli (correlation coefficient =  $0.770$ ,  $P < 0.0001$ ). The *Bacteroides/Prevotella* group also showed a significant reduction in percentage, from 24.2% for the low-frailty volunteers to 9.4% for the high-frailty volunteers ( $P = 0.05$ ).

For the number of lactobacilli, *F. prausnitzii*, and *Bacteroides/Prevotella* groups, it could be observed that the variation was the largest in the high-frailty volunteers. This might be explained by an increasingly unstable gut microbiota composition as frailty increases. The number of *Enterobacteriaceae* cells was  $6.6 \times 10^7$  cells/g (dry weight) in the low-frailty volunteers and  $4.7 \times 10^8$  cells/g (dry weight) in the high-frailty volunteers ( $P = 0.041$ ). Of the total number of cells, the percentage of *Enterobacteriaceae* was 0.05% in low-frailty volunteers and 0.3% in high-frailty volunteers ( $P = 0.041$ ).

The differences between fecal samples of elderly with low and high frailty may have several explanations. The GFI consists of components that can be expected to correlate with changes in the gut microbiota. A major component in the GFI is physical functioning. Due to low mobility, constipation may occur in volunteers. With altering fecal transit times, microbial niches may shift to different environmental conditions such as loss of contact with mucosa, depletion of energy substrates (29) or the increase of toxic compounds. Therefore it can be expected that some intestinal bacterial groups may increase in number while others may decrease (14). For some groups of bacteria different environmental conditions may result in less active cells yielding lower rRNA contents for fluorescent in situ hybridization measurements.

Another major GFI component is the psychosocial factor. Psychosocial stress has been suggested to be correlated with the gut microbiota (15). Host and gut microbiota form a complex ecosystem and as such, changes in gut microbiota composition may in its turn have implications for the health of the elderly host. Lactobacilli are involved in the stimulation of immune functions, aid in the digestion and/or absorption of food ingredients and minerals, and inhibit growth of exogenous or harmful bacteria (7). Several strains of *Lactobacillus* are mentioned as being beneficial to elderly health upon oral consumption in several studies (14).

*Bacteroides* is one of the five numerically predominant genera and plays an important role in the colonic ecosystem (26). The major part of polysaccharide digestion that occurs in the large intestine is accounted for by *Bacteroides* spp., which are known to ferment a wide variety of carbohydrates. Alterations in the numbers of such a nutritionally important bacterial group may affect bacterial metabolism and the complex colonic ecosystem of cross-feeding species (3). Colonic strains of *Bacteroides* have also been reported to produce bacteriocins.



In addition, some colonic *Bacteroides* are opportunistic pathogens (26).

*F. prausnitzii* is another abundant group which consists mostly of strains that are difficult to culture or unculturable and therefore at present the knowledge about this group is limited (4, 32). *F. prausnitzii* plays a role in carbohydrate breakdown and fermentation in the large intestine and is an important producer of butyrate, its main carbohydrate fermentation product. Butyrate plays a role in providing protection against colorectal cancer and ulcerative colitis. *Enterobacteriaceae* are potential pathogens that may cause autogenous infections when host resistance is reduced.

It is difficult to compare our results with other elderly gut microbiota results since the frailty status of the elderly in those studies was not established. The present study could confirm a not-significant reduction in bifidobacteria that has been reported with ageing (14) and an increase in the percentage of the *Lactobacillus/Enterococcus* group (23, 13) compared to younger adults. Bartosch et al. (3) characterized bacterial communities in feces from healthy elderly volunteers and hospitalized elderly patients by real-time PCR. They found a marked reduction of the mean 16S rRNA gene copy numbers of the *Bacteroides/Prevotella* group following hospitalization. Additionally, reductions in the total number of bacterial 16S rRNA genes and mean 16S rRNA gene copy numbers of bifidobacteria and *F. prausnitzii* in the hospitalized patients were reported. In contrast to this, the mean 16S rRNA gene copy numbers of enterobacteria and *Enterococcus faecalis* increased in these patients. If low frailty is regarded as analogous to healthy and high frailty to hospitalized, then these results are consistent with our findings.

Our results may have interesting implications. Probe sets may be used as sensitive tools in the analysis of gut microbiota composition in relation to human health and disease. Some groups of bacteria, like lactobacilli, might serve as indicator organisms for health monitoring. It is difficult to interpret the observed differences in bacterial composition and directly link them to frailty components. Whether modulation of gut microbiota composition (14, 20, 33) will have an effect on the development of frailty or components thereof remains to be investigated.

We especially thank the staff of the Heymans Elderly Centre, Groningen, for their cooperation and technical contribution to this project and the elderly volunteers for providing stool samples and data. We thank W. Sluiter for statistical advice. We also thank G. C. Raangs, A. C. M. Wildeboer-Veloo, T. He, and R. H. J. Tonk for their useful suggestions and technical assistance.

#### REFERENCES

- Abbott A. 2004. Gut reaction. *Nature* 427:284–286.
- Amann, R. I., B. J. Binder, R. J. Olson, S. W. Chisholm, R. Devereux, and D. A. Stahl. 1990. Combination of 16S rRNA-targeted oligonucleotide probes with flow cytometry for analyzing mixed microbial populations. *Appl. Environ. Microbiol.* 56:1919–1925.
- Bartosch, S., A. Fite, G. T. Macfarlane, and M. E. T. McMurdo. 2004. Characterization of bacterial communities in feces from healthy elderly volunteers and hospitalized elderly patients by using real-time PCR and effects of antibiotic treatment on the fecal microbiota. *Appl. Environ. Microbiol.* 70:3575–3581.
- Duncan, S. H., G. L. Hold, H. J. Harmsen, C. S. Stewart, and H. J. Flint. 2002. Growth requirements and fermentation products of *Fusobacterium prausnitzii*, and a proposal to reclassify it as *Faecalibacterium prausnitzii* gen. nov., comb. nov. *Int. J. Syst. Evol. Microbiol.* 52:2141–2146.
- Finegold, S. M., V. L. Sutter, and G. E. Mathisen. 1983. Normal indigenous intestinal flora, p. 3–31. In D. J. Hentges (ed.) *Hum. Intestinal microflora in health and disease*. Academic Press, New York, N.Y.
- Franks, A. H., H. J. M. Harmsen, G. C. Raangs, G. J. Jansen, F. Schut, and G. W. Welling. 1998. Variations of bacterial populations in human feces measured by fluorescent in situ hybridization with group-specific 16S rRNA-targeted oligonucleotide probes. *Appl. Environ. Microbiol.* 64:3336–3345.
- Gibson, G. R., and M. B. Roberfroid. 1995. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. *J. Nutr.* 125:1401–1412.
- Guarner F., and J.-R. Malagelada. 2003. Gut flora in health and disease. *Lancet* 361:512–519.
- Harmsen, H. J. M., P. Elfferich, F. Schut, and G. W. Welling. 1999. A 16S rRNA-targeted probe for detection of lactobacilli and enterococci in fecal samples by fluorescent *in situ* hybridization. *Microb. Ecol. Health Dis.* 11:3–12.
- Harmsen, H. J. M., A. C. Wildeboer-Veloo, G. C. Raangs, A. A. Wagendorp, N. Klijn, J. G. Bindels, and G. W. Welling. 2000. Analysis of intestinal flora development in breast-fed and formula-fed infants by using molecular identification and detection methods. *J. Pediatr. Gastroenterol. Nutr.* 30:61–67.
- Harmsen, H. J. M., A. C. M. Wildeboer-Veloo, J. Grijpstra, J. Knol, J. E. Degener, and G. W. Welling. 2000. Development of 16S rRNA-based probes for the *Coriobacterium* group and the *Atopobium* cluster and their application for enumeration of *Coriobacteriaceae* in human feces from volunteers of different age groups. *Appl. Environ. Microbiol.* 66:4523–4527.
- Harmsen, H. J. M., G. C. Raangs, T. He, J. E. Degener, and G. W. Welling. 2002. Extensive set of 16S rRNA-based probes for detection of bacteria in human feces. *Appl. Environ. Microbiol.* 68:2982–2990.
- He, T., H. J. M. Harmsen, G. C. Raangs, and G. W. Welling. 2003. Composition of faecal microbiota of elderly people. *Microb. Ecol. Health Dis.* 15:153–159.
- Hebuterne, X. 2003. Gut changes attributed to ageing: effects on intestinal microflora. *Curr. Opin. Clin. Nutr. Metab. Care* 6:49–54.
- Holdeman, L. V., I. J. Good, and W. E. C. Moore. 1976. Hum. fecal flora: variation in bacterial composition within individuals and a possible effect of emotional stress. *Appl. Environ. Microbiol.* 31:359–375.
- Hopkins, M. J., R. Sharp, and G. T. MacFarlane. 2001. Age and disease related changes in intestinal bacterial populations assessed by cell culture, 16S rRNA abundance, and community cellular fatty acid profiles. *Gut* 48:198–205.
- Hopkins, M. J., and G. T. MacFarlane. 2002. Changes in predominant bacterial populations in human faeces with age and with *Clostridium difficile* infection. *J. Med. Microbiol.* 51:448–454.
- Jansen, G. J., A. C. M. Wildeboer-Veloo, R. H. J. Tonk, A. H. Franks, and G. W. Welling. 1999. Development and validation of an automated, microscopy-based method for enumeration of groups of intestinal bacteria. *J. Microbiol. Methods* 37:215–221.
- Kiecolt-Glaser, J. K., L. McGuire, T. F. Robles, and R. Glaser. 2002. Psychoneuroimmunology: psychological influences on immune function and health. *J. Consult. Clin. Psychol.* 70:537–547.
- Kleessen, B., B. Sykura, H. J. Zunft, and M. Blaut. 1997. Effects of inulin and lactose on fecal microflora, microbial activity, and bowel habit in elderly constipated persons. *Am. J. Clin. Nutr.* 65:1397–1402.
- Langendijk, P. S., F. Schut, G. J. Jansen, G. C. Raangs, G. R. Kamphuis, M. H. F. Wilkinson, and G. W. Welling. 1995. Quantitative fluorescence *in situ* hybridization of *Bifidobacterium* spp. with genus-specific 16S rRNA-targeted probes and its application in fecal samples. *Appl. Environ. Microbiol.* 61:3069–3075.
- Manz, W., R. Amann, W. Ludwig, M. VanCanneyt, and K. H. Schleifer. 1996. Application of a suite of 16S rRNA-specific oligonucleotide probes designed to investigate bacteria of the phylum *Cytophaga-Flavobacter-Bacteroides* in the natural environment. *Microbiology* 142:1097–1106.
- Mitsuoka, T. 1992. Intestinal flora and ageing. *Nutr. Rev.* 50:438–446.
- Poulsen, L. K., T. R. Licht, C. Rang, K. A. Krogfelt, and S. Molin. 1995. Physiological state of *Escherichia coli* BJ4 growing in the large intestines of streptomycin-treated mice. *J. Bacteriol.* 177:5840–5845.
- Rockwood K., D. B. Hogan, and C. MacKnight. 2000. Conceptualisation and measurement of frailty in elderly people. *Drugs Aging* 17:295–302.
- Salyers, A. A. 1984. *Bacteroides* of the human lower intestinal tract. *Annu. Rev. Microbiol.* 38:293–313.
- Saunier, K., and J. Doré. 2002. Gastrointestinal tract and the elderly: functional foods, gut microflora and healthy ageing. *Dig. Liver Dis.* 34:19–24.
- Schuermans, H., N. Steverink, S. Lindenberg, N. Frieswijk, and J. P. J. Slaets. 2004. Old or frail: what tells us more? *J. Gerontol. A Biol. Sci. Med. Sci.* 59:M962–965.
- Sghir, A., G. Gramet, A. Suau, V. Rochet, P. Pochart, and J. Doré. 2000. Quantification of bacterial groups within human fecal flora by oligonucleotide probe hybridization. *Appl. Environ. Microbiol.* 66:2263–2266.

30. Steverink, N., J. P. J. Slaets, H. Schuurmans, and M. Van Lis. 2001. Measuring frailty. Development and testing of the Groningen Frailty Indicator (GFI). *Gerontologist* **41**:236–237.
31. Suau, A., R. Bonnet, M. Sutren, Godon J.-J., G. R. Gibson, M. D. Collins, and J. Doré. 1999. Direct analysis of genes encoding 16S rRNA from complex communities reveals many novel molecular species within the human gut. *Appl. Environ. Microbiol.* **65**:4799–4807.
32. Suau, A., V. Rochet, A. Sghir, G. Gramet, S. Brewaeys, M. Sutren, L. Rigottier-Gois, and J. Doré. 2001. *Fusobacterium prausnitzii* and related species represent a dominant group within the human fecal flora. *Syst. Appl. Microbiol.* **24**:139–145.
33. Sullivan Å., R. Bennet, M. Viitanen, A.-C. Palmgren, and C. E. Nord. 2002. Influence of *Lactobacillus* F19 on intestinal microflora in children and elderly persons and impact on *Helicobacter pylori* infections. *Microb. Ecol. Health Dis.* **3**:17–21.
34. Vollaard, E. J., and H. A. L. Clasener. 1994. Colonization resistance. *Antimicrob. Agents Chemother.* **38**:409–414.
35. Wilson, K. H., and R. B. Blitchington. 1996. Human colonic biota studied by ribosomal DNA sequence analysis. *Appl. Environ. Microbiol.* **62**:2273–2278.
36. Zoetendal, E. G., A. D. L. Akkermans, and W. M. De Vos. 1998. Temperature gradient gel electrophoresis of 16S rRNA from human fecal samples reveals stable and host-specific communities of active bacteria. *Appl. Environ. Microbiol.* **64**:3854–3859.
37. Zoetendal, E. G., K. Ben-Amor, H. J. M. Harmsen, F. Schut, A. D. L. Akkermans, and W. M. De Vos. 2002. Quantification of uncultured *Ruminococcus obeum*-like bacteria in human fecal samples by fluorescent in situ hybridization and flow cytometry using 16S rRNA-targeted probes. *Appl. Environ. Microbiol.* **68**:4225–4232.